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50. Internationales Wissenschaftliches Kolloquium
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J. H. AGARWAL and VIJAYA AGARWAL

Potential Applications of Chip-based Systems for Management of Quality and Food Safety in Dairy Processing Industry

ABSTRACT

QUALITY AND SAFETY OF PRODUCTS, CONFORMING TO LEGISLATIVE regulations, is an important consideration for dairy processing industry. Time-consuming and laborious techniques involving wet-chemistry are currently used for monitoring the product quality on off-line basis. Regulatory agencies and dairy processing industry need fast, cost-effective, on-line and automated methods that are accurate and reliable for rapid and frequent monitoring and quality control of raw materials and products at various stages of a production process.

The chip-based technology, using the miniaturized electronic mechanics (MEM), has a great potential for quality monitoring of a product in dairy processing. Unlike conventional quality and process control systems, the technology facilitates low-cost device-level integration of on-line measurements, quality monitoring and process control functions as the microprocessor and microcontroller components are embedded in to the chips.

The widespread use of antibiotics and chemotherapeutics to control diseases and improve animal performance in dairy parlors has led to the occurrence of veterinary drugs and chemical residues in dairy products. Many countries have introduced more restrictive control measures but traditional microbial and test methods are not sensitive enough to meet new regulations and classical analytical techniques are often precluded owing to the

level of experience, skills and cost required. Biosensors, biochips and biochip-arrays technologies, based on a concept to use the chip as a reaction platform with single or multiple specific antibodies attached on the surface, offer alternative methods to detect and measure concentrations of residues and contaminants (such as pesticides, antibiotics, growth promoters, veterinary drugs, pathogenic bacteria and their toxins). These methods are highly sensitive, do not require elaborate sample preparation, and can be rapidly carried out on-line at a low cost. Besides, the biosensors can also be used in the detection of metabolic levels in veterinary testing and animal husbandry, for example estrus detection by monitoring progesterone levels in milk, on-set of spoilage in meats.

Some state-of-the-art devices are described and their scope in quality and food safety in dairy processing industry is discussed illustrating a few applications.

Keywords: *Biosensors, chip-based systems, instrumentation, on-line testing, quality and safety, dairy products, contaminants and residues.*

1. 0 BIOSENSORS, BIO-CHIPS AND BIOCHIP-ARRAYS

1.1 Biosensor

A BIOSENSOR IS A COMPACT ANALYTICAL DEVICE INCORPORATING A biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer. The usual aim of a biosensor is to produce either discrete or continuous digital electrical signals which are proportional to single analyte or a related group of analytes [1]. The main components of a biosensor are: a biological sensing element, a transducer, a signal conditioner, a data processor and a data display. The biocatalyst converts the substrate to product. The transducer that converts it to an electrical signal determines this reaction. The key part of a biosensor is the transducer, which makes use of a physical change accompanying the reaction. The reaction, typically, may be heat absorption or generation as in calorimetric biosensors, changes in distribution of charges causing a electrical potential to be produced (potentiometric biosensors), movement of electrons produced in a redox reaction (amperometric biosensensors), effects due to mass of the reactants or products (piezo-electric and microcantilever biosensors), generation or absorption of optical radiation during the reaction (optical biosensors).

1.2 Biochip

A biochip is a solid subtract with multiple specific ligands - such as antibodies, antigens – attached at pre-defined sites on the surface. The chip utilizes the basic principle of immunology. In a typical biochip [2], up to about 25 tests are pre-fabricated onto the biochip surface. With advancement in technology, the capacity of tests per chip is on increase.

1.3 Biochip-Array

The technology is based on the concept to use a biochip as a reaction platform, with multiple specific ligands. After addition of a sample to the biochip, analytes present in the sample bind to specific ligands and generate corresponding chemi-luminescent signals for the measurements.

Dedicated user-friendly software(s) control all operations from onboard maintenance, to calibration validation, to automatic system initialization, to sample profiling for a variety of tests with a facility to pre-defined cut-off values for semi-quantitative measurements – a feature desirable for on-line quality and process control in production processes.

1.4 Microprocessors and Microcontrollers Embedded Chip-based Systems

Unlike conventional systems for quality and process controls in production units, the chip-based systems facilitate cost-effective device-level integration of on-line measurements, quality monitoring and process control functions. For this purpose, the microprocessor and microcontroller components are embedded into the chips.

Conventional modular systems used for quality control functions in production processes are with a sensing module, a processing module (usually a microcomputer or a computer) and a control module that are separately housed and functionally wired to each other. In chip-based systems, these modules can be easily integrated. Analog signals from the transducer after signal amplification and noise filtration are converted to digital signals and passed to a microprocessor where the data is processed. The signal amplifier, noise filtering unit and the microprocessor are embedded in the chip itself. A microcontroller can be added into the chip for control functions. The integrated package – the sensor, associated electronic circuit, the microprocessor and the microcontroller – acts as an on-line quality control device by which certain parameters related to quality of the product can be monitored and controlled within the pre-specified limits. In further developments, a wireless circuit is added to the chip for non-contact interface between the chip and the external equipment.

2.0 QUALITY MONITORING OF MILK

2.1 Measurement of Progesterone

Estrus detection is a financial concern in reproductive management of dairies, as missed estrus is a main cause of lost income. A biosensor reported by Claycomb and Delwiche [3] was employed for measurement of progesterone in bovine milk and detection of estrus. The sensor was designed to operate on-line using microinjection pumps and valves for fluid transport, fiber optics and photodiodes for light measurement, and a control computer to control the sequencing of operation.

2.2 IGF-1 Detection in Milk

Recombinant bovine somatotropin (rBST) treatment is adopted in dairy parlors to augment cows' milk yield. Insulin-like growth factor-1 (IGF-1), a suspected carcinogen, is present in milk from cows treated with rBST. Its presence in milk for human consumption is potentially a health hazard. A surface plasmon resonance-based biosensor system was developed [4] for evaluation of IGF-1 in cows' milk. The features of the technology – fully automated, measures in real time, and sharpened yes/no response offer several advantages compared to conventional enzyme-linked immunoassay (ELISA).

2.3 Fat, Protein, Lactose, Urea, SNF, Bacteria and Somatic Cells in Milk

and Milk Products

Semi-automatic, off-line detection and measurements on components and constituent values of fat, protein, lactose, SNF, urea, individual bacteria (such as bacillus, lactobacillus, pseudomonas, bacterium) and somatic cells are performed by near-infrared techniques and flow cytometry [5]. Flow cytometry is a method to measure various biological parameters of cells by first treating them with light absorbing or fluorescing compounds and then passing them through a narrow fluid stream that is interrogated with a laser beam. These instruments are computer controlled and software driven. With some modifications and employing auto-sampling attachments, the systems can be adopted for on-line working, however at higher costs compared to that of chip based systems.

2.4 Urea in Milk

A potentiometric biosensor, in which the bio-component part was an urease coupled to an ammonium ion selective electrode of a transduder, was developed for measurement of urea concentrations in milk samples. Response time was low, typically 2 min. Emerging concern of the presence of urea in milk, in particular in “synthetic milk”, necessitated the development of the technique [6].

2.5 Pesticide residue in milk

In immunoassay-based pesticide detection systems, the enzyme linked immunosorbent assay (ELISA) combines selective antibodies with sensitive enzyme reactions to produce analytical systems capable of detecting very low levels of pesticides. The immunochemical reaction contributes high selectivity due to the extraordinary discriminatory capability of antibodies. The powerful catalytic ability of the enzyme provides highly sensitive detection [7].

A rapid immunoassay kit for pesticide detection [8] utilizes micro-sized magnetic particles as solid support. Detection and quantification of a pesticide in a sample involves three steps. Step 1: The sample to be analyzed, an enzyme conjugate (a pesticide labeled with an enzyme), and the magnetic particles with attached antibodies are added to a disposable test tube. Incubation for 15-20 minutes is carried out. Any pesticide present in the sample and the pesticide in the enzyme conjugate compete for the limited number of antibody binding sites on the magnetic particles. Step 2: A magnetic field is applied to the test tube. All particles are pulled and held to the tube wall while unbound reagents are decanted. Particles are washed twice. Step 3: A colour reagent is added to develop a coloured immunocomplex in which the colour intensity is inversely proportional to the concentration of the pesticide in the sample. The colour intensity is measured spectrophotometrically. A microprocessor-based analyzer automatically converts immunoassay optical readings to sample concentrations. Programs for various assay methods are stored along with the calibration data for convenient and instantaneous operation.

The kit provides highly sensitive detection. Typical detection ranges for some of the pesticides in liquid samples are: 0.05 to 5.0 ppb (parts per billion) for alachlor, 0.25 to 100 ppb for aldicarb, 0.046 to 5.0 ppb for atrazine, 0.1 to 5.0 ppb for carbendazim, 0.25 to 3.0 ppb for carbaryl, 0.06 to 5.0 ppb for carbofuran, 0.04 to 3.0 ppb for cyanazine, 0.7 to 50 ppb for 2,4-D, 0.05 to 5.0 ppb for metolachlor, and 0.02 to 5.0 ppb for paraquat.

Specific immunoassays, using an amperometric biosensor, for detection of 2.4-D and 2,4,5-T herbicides were developed [9]. The total time for assay was less than 20 min. The ranges of detection of 2,4-D and 2,4,5-T were $1x10^{-11}$ to $5x10^{-7}$ M and $5x10^{-11}$ to $5x10^{-7}$ M, respectively. For determination of 2,4-D in milk, the method was applied without a sample pre-treatment.

A biosensor-based pesticide detection technique, employing surface plasmon resonance and using real-time biospecific interaction analysis, is reported for detection of pesticides [10]. The biospecific interface was a sensor chip to which a derivative of atrazine had been covalently bound. Monoclonal antibodies against atrazine were mixed with the sample containing herbicide, and then the solution reacted with the biospecific interface. As the interaction between free antibodies and the immobilized derivative bound to the surface proceeded, the surface plasmon resonance response changes inversely to the concentration of atrazine. A detection limit of 0.05 ppb of atrazine in sample was reached. The analysis time was about 15 minutes, and after each measurement regeneration of the sensor chip was carried out. In some recent developments, plastic disposable biosensors and piezoelectric crystal biosensors with surface modification are being experimented and perfected for detection of pesticides in liquid samples.

2.6 Detection of Toxins

Under an USDA-ARS National Program on Food Safety, a biosensor-based immunoassay using suface plasmon resonance has been developed [11] to detect *Staphylococcus auresus* enterotoxin A and B in food samples such as milk, meats. These toxins cause gastroenteritis. The toxin molecules in the sample bind to the sensor surface, and the refractive index at the surface changes. The time it takes for a response from the interaction provides a measure of how much toxin is actually present in the sample. The method can detect multiple bacterial toxins in a sample. Detection level is of the order of 10 ppb of toxin per gram of sample.

2.7 Detection and Assays for Bacteria

Test kits, based on adenosine triphosphate (ATP) bioluminescence, for on-site, same day detection and assays of food-borne pathogens *E. coli O157, Listeria* and *Salmonella* are now available [12, 13]. The fastest testing laboratory procedures currently in use require at least 24 hours delivering the results, at which point the products are to be stored awaiting clearance

3.0 QUALITY MONITORING OF MEATS

3.1 Meat Freshness Assessment

For rapid assessment of meat freshness, a biosensor-array was employed to measure glucose concentration at depths of 2 and 4 mm below the meat surface [14]. The results indicated that *in situ* assessment of complex food conditions such as microbial or oxidative spoilage, progressive fermentation is feasible by using a knife-probe biosensor-array that provides glucose depth profiling close to the surface.

3.2 Detection of Meat Spoilage and Aging

A sensor composed of Ag/AgCl electrode and a platinum electrode on which putrescine or xanthine oxidase were immobilized was used to monitor meat quality to estimate bacterial spoilage and progress of aging [15]. The method was based on potential-step chrono-amperometric technique in which the potential applied was stepped from 300 mV to 600 mV. The results indicated that with some modifications and further refinements the method can be used for the estimation of meat quality during aging.

In another approach [16], a two-line flow injection analysis (FIA) biosensor was used for simultaneous detection of bacterial spoilage and the progress of aging. The sensor was applied to a vacuum packed meat stored at 0.5 and 10 deg C. The results indicated that the progress of aging could be monitored in the entire (0.5 - 10 deg) temperature range, and the bacterial spoilage could be detected before the appearance of putrid odour at 5 and 10 deg C. Thus the FIA biosensor is useful for quality control of meat aging at 5 and 10 deg C, but not at 0 deg C.

3.3 Electronic Olfaction for Spoilage Detection

For dairy processing and food industry, electronic olfaction devices (e-noses), are being experimented to sense bacterial contamination and resulting spoilage in products. A typical e-nose has two major components: a sensor that detects odours and a set of electronic circuit elements that interprets the resulting signals. The sensor incorporates materials like metal oxides or advanced polymers. When exposed to certain volatile compounds or combinations of them, the sensor material changes its size, colour, or electrical resistance. By using several different materials in an array of sensors mounted on a single chip, an e-nose is made to sense multi-odours [17, 18].

3.4 Molecularly Imprinted Polymers for Meat Freshness

One of the markers of meat spoilage is the decarboxylation of free amino acids in meat by enzymes released by spoilage micro-organisms. Presence of two of these products, putrescence (1,4-diaminopentane) and cadaverine (1,5-diaminopentane), correlate well with surface bacteria counts. Molecularly imprinted polymers (MIPs), a class of synthetic polymers that are tailored to selectively detect a particular substance, undergo a detectable colour change when brought in contact with biogenic amines (such as putrescence, cadaverine). This property is used in evaluation of meat freshness. MIPs can be incorporated into meat containers or employed in fiber optic detection devices to detect probable spoilage of meat within the container.

4.0 DRUG RESIDUE IN MEATS, MILK AND MILK PRODUCTS

Drug-use plays an integral part of modern dairying. Some veterinary drugs were originally introduced to improve animal health and enhance their performance; for example, anabolic steroids (testosterone, trenbolone) and B-agonists (clenbuterol, brombuterol) – to increase the quality and efficiency of meat production; corticosteroids – for cattle fattening; oestrogenic hormones (stillbenes, zerol) – for growth promotion in cattle; antibiotics (chloramphenicol, sulphadiazine) – for treatment of diseases (pneumonia, rhinitis, mastitis). Many such drugs accumulate in animal tissue and enter the food chain for human consumption once the animal is slaughtered for meat or through other products of dairies. These drugs can have detrimental effects on human. As the use of drugs can not be totally prevented, it has become necessary to take measures to protect the consumers from the effect of drug residues in food.

Sensitive and cost effective methods of monitoring drug residue in dairy products are essential to ensure that residue levels remain within the permitted regulatory limits. Chromatographic methods like gas chromatography – mass spectrometry (GC-MS), LC-MS have been used to monitor drug residue levels. Some diagnostic kits employ ELISA technology for the measurements. An ELISA based commercially available kit [19] can be fully automated. The kit has certain advantages over the conventional GC-MS; such as: 5 to 10 times greater sensitivity, ready to use support for sample pre-treatment, faster operation (a throughput of up to 120 samples in 2-3 hours), lower equipment cost and easy-to-use.

5.0 CONCLUDING REMARKS

Pressure on the dairy processing industry to bring quality and safe products to market has never been so intense. With standards in food safety continually on the rise and increasingly attentive consumers to satisfy, the technology to monitor product quality plays a vital role. Biosensors and chip-based systems show a great potential for applications in the dairy processing industry, particularly where rapid, low cost, high sensitivity and specificity measurements in on-line working are required. The technology, however, must overcome several obstacles before becoming a commercial success. Some of these are: faster transfer of technology from lab to industry, production and availability of reliable and inexpensive sensors specific to dairy product quality monitoring, and integration of the technology in the existing dairy processing systems.

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Appendix 'A' lists some more references on topics related to quality and food safety.

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He is a pioneer in the field of applications of Electronics, Microprocessors, Computers and Information Technology in Agriculture. In 1970s, associated as an Expert with the Electronics Commission of India and the Scientific Advisory Committee to the Cabinet, Govt. of India, he introduced the Applications of Electronics and allied technologies in Agriculture in India. Thereafter, he has been guiding a large number of programmes under the ICAR, CSIR, DST, DOE, and several Universities. During 1985 to 1995, Dr. Agarwal headed a prestigious project of UNDP and Govt. of India on "Applications of Microprocessors and Computers in Agriculture". Under the Project and deputed by the UNDP and GOI, he visited several countries (USA, UK, Australia, Germany, Japan, China, etc.) on technical missions.

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Prof. Agarwal has been the President of the Engineering Sciences Section, Indian Science Congress during 1993-94. She served as a Member of the National Executive Committee and the National Council of the Indian Science Congress for several terms. She is also actively associated with the Indian Women Scientists' Association and contributing in their scientific activities. Countries visited : Austria, Canada & USA.

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APPENDIX 'A'

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